Malaysian Journal of Science **29** (Special Issue): 11 – 18 (2010)

Isolation, Detection and Genomic Differentiation of *Escherichia coli* from Aquatic Environments in Kelantan, Malaysia

Rathi, A.[1, 2], Thong Kwai Lin[1, 2]* and Chong, V. C.[1]

[1]Institute of Biological Sciences, Faculty of Science & [2]BioMedical Science and Molecular Typing Laboratory, Institute of Postgraduate Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia.
* thongkl@um.edu.my (corresponding author)
Received on 24th April 2009, accepted in revised form 27th September 2009.

ABSTRACT The main aims of this study were to isolate and detect the presence of pathogenic *E. coli* in selected aquatic environments in Bachok, Kelantan, as well as to determine the presence of their virulence genes and the genomic diversity among the isolates. Fifty water samples from various aquatic environments of Bachok, Kelantan were examined for total coliform and pathogenic *E. coli*. The presence of total coliform was significantly correlated to *E. coli* ($p<0.05$). Among the putative *E. coli*, 176 isolates retrieved from selective media, 116 (66%) from 29 water samples were positive for biochemical tests and harbored the *phoA* gene, which is the housekeeping gene for *E. coli*. A hexaplex PCR was performed to detect six virulence genes in pathogenic *E. coli* such as heat-stable toxin 1 (ST1), heat-labile toxin 1 (LT1), heat-labile toxin 2 (LT2), verotoxin1 (VT1), verotoxin 2 (VT2) and attachment and effacement (*eae*A). Isolates from only one sample harbored the ETEC as the isolates were positive for the LT1-heat labile toxin. Antimicrobial susceptibility tests showed that only ETEC isolates was resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. The rest of *E. coli* isolates were susceptible to the tested antibiotics. The analysis of genomic diversity of 116 *E. coli* isolates by Repetitive Extragenic Palindromic (REP)-PCR generated 27 patterns (F=0.26-1.0). The REP-PCR profiles were reproducible and the multiple DNA fingerprints showed that the *E. coli* isolates were genetically diverse. A dendrogram generated by the UPGMA algorithm showed 4 clusters of *E. coli* isolates based on 80% similarity. Overall, REP-PCR generated high genetic variability within the *E. coli* isolates. REP-PCR is a promising molecular method for determining the genomic diversity of environmental *E. coli* strains.

ABSTRAK Kajian ini bertujuan untuk mengesan kehadiran patogenik *E. coli* dalam persekitaran air terpilih di Bachok, Kelantan, serta kehadiran gen-gen virulen and diversiti genom sesama isolat *E. coli*. Lima puluh sampel air dari pelbagai persekitaran perairan Bachok, Kelantan telah diperiksa bagi menentukan kualiti mikrobiologi dengan menggunakan kaedah fenotip dan genotip untuk pengesanan jumlah koliform dan *E. coli*. Kehadiran jumlah koliform adalah dihubung kait nyata sekali kepada *E. coli* ($p<0.05$). Antara 176 isolat yang dikesan, 116 isolat (66%) *E. coli* adalah positif berdasarkan ujian biokimia dan kehadiran gen *phoA* yang merupakan gen 'housekeeping' untuk *E. coli*. Ujian heksapleks PCR dijalankan untuk mengesan enam gen virulen dalam *E. coli* patogenik seperti 'heat-stable toxin 1' (ST1), 'heat-labile toxin 1' (LT1), 'heat-labile toxin 2' (LT2), 'verotoxin1' (VT1), 'verotoxin 2' (VT2) dan 'attachment and effacement' (*eae*A). *E. coli* daripada hanya satu sampel (EC15) mengandungi gen LT1 yang mengekodkan toksin `heat-labile'. Hanya ETEC menunjukan ketahanan terhadap ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole dalam ujian sensitiviti antibiotik. Isolat *E. coli* yang lain tidak menunjukkan sebarang ketahanan terhadap antibiotik yang diuji. Analisis diversiti genom isolat *E. coli* oleh Repetitive Extragenic Palindromic (REP)-PCR menghasilkan 27 profil (F=0.26-1.0). Profil REP-PCR mampu dihasilkan semula dan fingerprint DNA menunjukkan bahawa isolat *E. coli* mempunyai diversiti yang tinggi. Dendrogram yang dihasilkan oleh algoritma UPGMA menunjukkan 4 cluster isolat *E. coli* berdasarkan 80% persamaan. Keseluruhannya, REP-PCR menghasilkan variasi genetik yang tinggi dalam isolat *E. coli*. REP-PCR adalah satu kaedah molekul yang berpotensi untuk penentuan diversiti genom *E. coli* dari alam sekitar.

(**Keywords:** *Escherichia coli*, PCR, pathogenic, diversity, REP-PCR)

INTRODUCTION

Microbial pollution of water is a growing environmental and public health crisis worldwide. Fecal contamination from humans and animals is believed to be a major cause for increased microbiological and nutrient loads in coastal and inland waterways [1]. Microbial contamination in waterways can originate from various sources such as wastewater treatment outfalls, municipal waste and

discharge from large-animal feeding operations. Indicator microorganisms are used to predict the presence of the potential risk associated with pathogenic microbes and total coliforms have been used extensively for many years as indicators for determining the sanitary quality of surface waters [2].

Diarrhea caused by pathogenic *Escherichia coli* is one of the main diseases associated with contaminated water supply and poor sanitation. *E. coli* is a gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* strains are harmless, but pathogenic strains can cause serious disease such as neonatal meningitis, diarrheal disease, and gram-negative pneumonia [3]. Six major pathogenic *E. coli* strains are enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EaggEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) [4].

In Malaysia, the active promotion of environmental sanitation has improved the health of the population, with coverage reaching almost 100% for all states with the exception of Kelantan and Sabah [5]. In the present study, waterways around Bachok, Kelantan were studied. Bachok is a territory and town in Kelantan. It is a rural area approximately 20 kilometers south east of Kota Bahru city. World Health Organization (WHO) representative in Malaysia (2004) reported a high incidence of poverty in Kelantan (12%) [6]. Besides that, a recent study reported high concentration of fecal coliform and *E. coli* in Kelantan river waters which led to high gastrointestinal diseases in Kelantan [7].

The aims of this study were to isolate and detect pathogenic *E. coli* in selected aquatic environments in Bachok, Kelantan, to determine the prevalence of their virulence genes and the genomic diversity among the isolates.

MATERIALS AND METHODS

Sources of isolates

A total of 50 water samples were collected from various waterways including river water (n = 39), sea water (n = 9) and waterfall (n = 2) in Bachok, Kelantan from June 16 to 18, 2008. Samples were collected in pre-sterilized bottles and were kept on ice and transported to the laboratory to be processed within 3 h.

Isolation and identification of *E. coli* and other coliforms

The membrane filtration method was carried out according to instruction by the United States Environmental Protection Agency (USEPA, 1986) to isolate *E. coli* and total coliforms from water samples [8]. In this procedure, water samples were filtered through a sterile, 47 mm diameter membrane (0.45-µm-pore-size gridded membrane filters [Sartorius, Germany]). After filtration, the membrane containing the bacteria was placed on a selective differential medium, Chromocult Coliform Agar (CCA, Merck, Germany) and incubated at 37°C for 24 h to detect presumptive *E. coli* and total coliforms [9]. After an overnight incubation, coliform colonies turned salmon-red and *E. coli* colonies turned dark blue or purple on this media. The colony counts of total coliform and *E. coli* were entered in Microsoft Excel spreadsheet and descriptive statistics were computed. Pearson correlation test was performed using SPSS, version 14.0. Presumptive colonies of *E. coli* from the CCA were re-cultured on another CCA plate to obtain distinct, characteristic colonies and then subcultured on non-selective medium, nutrient agar (Merck) incubated at 37°C for 24 h to obtain pure single *E. coli* isolates for further confirmation. Presumptive *E. coli* were subjected for further confirmation by using an array of biochemical tests such as Indole, Methyl red, Voges - Proskauer, Citrate and by the bacterial identification API 20E Kit (Biomerieux) [10].

Extraction of total DNA and PCR assay

Boiling method was used for the extraction of crude bacterial DNA as template for PCR assay. Single colonies from fresh *E. coli* cultures were transferred to a microfuge tube containing 50 µL 1× PBS pH 7.3 and centrifuged at 13,400 rpm for 2 min The supernatant was then discarded. The cell pellet was resuspended in 50 µL sterile water, boiled in thermal cycler (Perkin Elmer) at 99ºC for 5 min, and re-centrifuged at 13,400 rpm for 2 min. An aliquot (5 µL) was used for subsequent PCR analysis.

E. coli isolates that were determined by biochemical tests were further confirmed by PCR-based assay targeting the *phoA* gene, which is the housekeeping gene for *E. coli* [11]. The oligonucleotide primers used in this study and the size of expected PCR products are listed in Table 1. The reaction mixture consisted of 25 µL volumes containing 1 × PCR buffer (GoTaq® Flexi Buffer), 1 mM $MgCl_2$, 140 µM of each dNTP, 0.1µM concentrations of each primer, 0.5 U of *Taq* DNA polymerase (GoTaq® DNA Polymerase, Promega, USA) and 5 µL of crude bacterial DNA (~100ng). The reactions were

Table 1. Primers used for detection of housekeeping gene (monoplex) and virulence genes (multiplex) of *E. coli*.

Target gene	Primers	Sequence	Expected band
Alkaline phosphatase (*phoA*)	Pho-F	GTC ACA AAA GCC CGG ACA CCA TAA ATG CCT	903bp
	Pho-R	TAC ACT GTC ATT ACG TTG CGG ATT TGG CGT	
Heat-stable toxin 1 (ST1)	ST1-F	CTT TCC CCT CTT TTA GTC AG	175bp
	ST1-R	TAA CAT GGA GCA CAG GCA GG	
Heat-labile toxin 1 (LT1)	LT1-F	TTA CGG CGT TAC TAT CCT CTC TA	275bp
	LT1-R	GGT CTC GGT CAG ATA TGT GAT TC	
Heat-labile toxin 2 (LT2)	LT2-F	ATA TCA TTT TCT GTT TCA GCA AA	720bp
	LT2-R	CAA TAA AAT CAT CTT CGC TCA TG	
Verotoxin 1 (VT1)	VT-F	GAA CGA AAT AAT TTA TAT GTG	523bp
Verotoxin 2 (VT2)	VT-R	CCT GAT GAT GGC AAT TCA GTA	523bp
Attachment and Effacement (eaeA)	AE22	ATT ACC ATC CAC ACA GAC GGT	397bp
	AE20-2	ACA GCG TGG TTG GAT CAA CCT	

performed in Mastercycler (Eppendorf). The PCR conditions included an initial denaturation at 94ºC for 2 min, followed by 35 cycles of denaturation at 94°C, 1 min, annealing at 56°C, 1 min and extension at 72°C, 1 min, and followed by a final extension step at 68°C for 1 min. Each reaction included a negative control (water as template) and positive controls that included DNA from known pathogenic strains of *E. coli*. PCR products were resolved on a 1.2% w/v agarose gel and electrophoresed for 45 min at 90 V. Each gel included a DNA molecular weight standard (100bp ladder, Promega Corporation USA). The ethidium bromide stained gel was visualized and photographed using the BioRad Molecular Imager (Gel Doc™ XR).

Multiplex PCR for toxin genes

A multiplex PCR was used to amplify the ST1, LT1, LT2, *eae*A and VT genes using primers as described previously [11]. Multiplex PCR assay was performed in 50 µL volumes containing 1 × PCR buffer (GoTaq® Flexi Buffer), 3 mM $MgCl_2$, 200 µM of each dNTP, primer concentration ranged between 0.07 µM and 0.7 µM, 1.5 U of *Taq* DNA polymerase (GoTaq® DNA Polymerase, Promega) and 5 µL of bacterial DNA. Both monoplex and multiplex PCR assays employed the same amplification and electrophoretic conditions. Bacterial strains used as positive control strains were *E. coli* SA53 (LT2 and VT positive), *E. coli* ATCC 35401 (LT1 and ST positive) and *E. coli* 0157(VT and *eae*A).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by an agar diffusion disk method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (formerly known as National Committee for Clinical Laboratory Standards [12]. Antimicrobial agents used in the study were ampicillin (10µg), chloramphenicol (30µg), trimethoprim-sulfmethaxzole (25µg), tetracycline (30µg), gentamycin (10µg), kanamycin (30µg), ciprofloxacin (5µg), nalidixic acid (30µg), streptomycin (10µg) and ceftriazone (30µg). *E. coli* ATCC 25922 was used as a reference strain for quality control. Culture broth was diluted in normal saline solution to a density of 0.5 McFarland turbidity standard and diluted broths were spread onto Mueller-Hinton agar plates using sterile cotton swab. After air drying for a min, antibiotic discs were aseptically placed onto the surface of bacterial lawn-agar and pressed to ensure complete contact of disc with the agar. The plates were incubated at 37°C for 24 h. The inhibition zones were measured and recorded. The breakpoints used to categorize isolates as resistant or susceptible to each antimicrobial agent were as those recommended by CLSI[12].

Repetitive extragenic palindromic (REP) - PCR analysis

REP - PCR was used to amplify repetitive elements from bacterial isolates to generate DNA fingerprint patterns. REP - PCR was carried out following the

method previously described by McLellan *et al*. with some modifications [13]. Briefly, the primer REP1R (III ICG ICG ICA TCA TCT GG) was used under the following conditions: An initial cycle of 5 min at 94°C, 5 min at 33°C, and 5 min at 68°C, followed by 30 cycles of 1 min at 94°C, 1 min at 40°C, and 2 min at 68°C, and a final extension of 16 min at 68°C. The reaction was prepared with 5 µL of DNA template (~100ng) and a negative control containing 5 µL of water as template. Separation of amplified DNA fragments was accomplished via horizontal gel electrophoresis by using 1.5% w/v agarose gel and run at 60 V for 5 h. A 1kb and 100bp size ladders (Promega Corporation USA) were used as DNA size markers. REP-PCR assays were repeated twice for all the isolates to determine reproducibility.

Cluster analysis

Cluster analysis of REP - PCR patterns were performed using GelCompar software (Applied Maths, Kortrijk, Belgium). Normalizations were done on the positions of bands on each gel by using the 1kb ladder from 300bp to 5000bp as an external reference standard. Multiple gels were then compared using the normalization with the same set of external standards. A dendrogram was constructed by using the unweighted pair group method with arithmetic means (UPGMA).

RESULTS AND DISCUSSION

Isolation and confirmation

Total coliforms were recovered from all the 50 water samples with an average density of 1×10^8 cfu/100mL. About 176 putative *E. coli* isolates were recovered from 44 samples with an average density of 1×10^6 cfu/100mL. No *E. coli* was isolated from 6 coastal water samples. Highest concentrations of *E. coli* (up to 5.2×10^8 cfu/100mL) were observed for river water samples and lowest in coastal water samples (1×10^2 cfu/100mL). Pearson correlations were used to determine relationships between the colony counts. The correlation coefficient for the regression comparing the count of total coliform and *E. coli* was 0.767. According to the results obtained, the presence of total coliform was significantly correlated to *E. coli* ($p<0.05$).

Of the 176 putative *E. coli* isolates recovered, 66% (116 isolates from 29 water samples) were confirmed *E. coli* based on biochemical tests and were positive for the housekeeping gene, *phoA*. In a recent study, *gad* gene was used as a marker for identifying *E. coli* isolated from various sources [14]. However the use of *gad* gene has limitation as other enteric bacteria particularly *Shigella* also carries the same gene. The selective medium, CCA used in this study was considered less suitable for direct detection of *E. coli* because of the lower *E. coli* counts and the high percentage (20%) of false-negative results found in this study. Putative *E. coli* isolates from CCA were classified as *Klebsiella* spp after biochemical tests. *Klebsiella* which is also a member of coliform group shares similar morphological and biochemical characteristics with *E. coli*. Thus, other media should be explored for future studies to simplify the screening procedure. Hengstler *et al.* [15] reported the diagnostic efficiency of CHROMagar™ *E. coli* media which reduced the number of false-positive colony picks.

Virulence gene detection

The hexaplex PCR assay was designed to detect six virulence genes in pathogenic *E. coli* (ST1, LT1, LT2, VT and *eae*A) and was applied on positively identified *E. coli* in this study. Four out of 116 *E coli* were positive for the LT1 gene which codes for LT heat-labile toxin. These four isolates were all from one water sample collected at the jetty area (Figure 1). None of the other genes was detected in the local *E. coli* isolates. Enterotoxigenic *E. coli* (ETEC) that produces LT1 toxin is an important cause of diarrhea in infants and travellers in underdeveloped countries or regions of poor sanitation. ETEC belongs to a heterogeneous family of lactose fermenting *E. coli* and has a wide variety of O antigenic types with colonization factors which allow the organism to readily colonize the small intestine and cause diarrhea [16]. Qadri *et al*. [16] had reported that ETEC is a major cause of diarrheal disease where drinking water and sanitation are inadequate and transmission may occur while bathing or using water for food preparation. These forms of transmission are common in areas where ETEC is endemic [16]. In the present study ETEC was detected in the sample collected near jetty, where there was a large community of foreign workers and sanitation could be compromised. Recently, Alhaj and coworkers [17] successfully detected STEC and EPEC strains from marine samples using a single multiplex PCR reaction.

Antibiograms analysis

On the basis of antibiotic susceptibility tests, only ETEC isolates were multiple resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. The rest of the *E. coli* isolates were susceptible to all tested antibiotics. Multidrug resistance is increasing in ETEC due to widespread use of chemotherapeutic agents [16]. Sayah *et al.* [18] reported that *E. coli* isolates from domestic species were resistant to larger

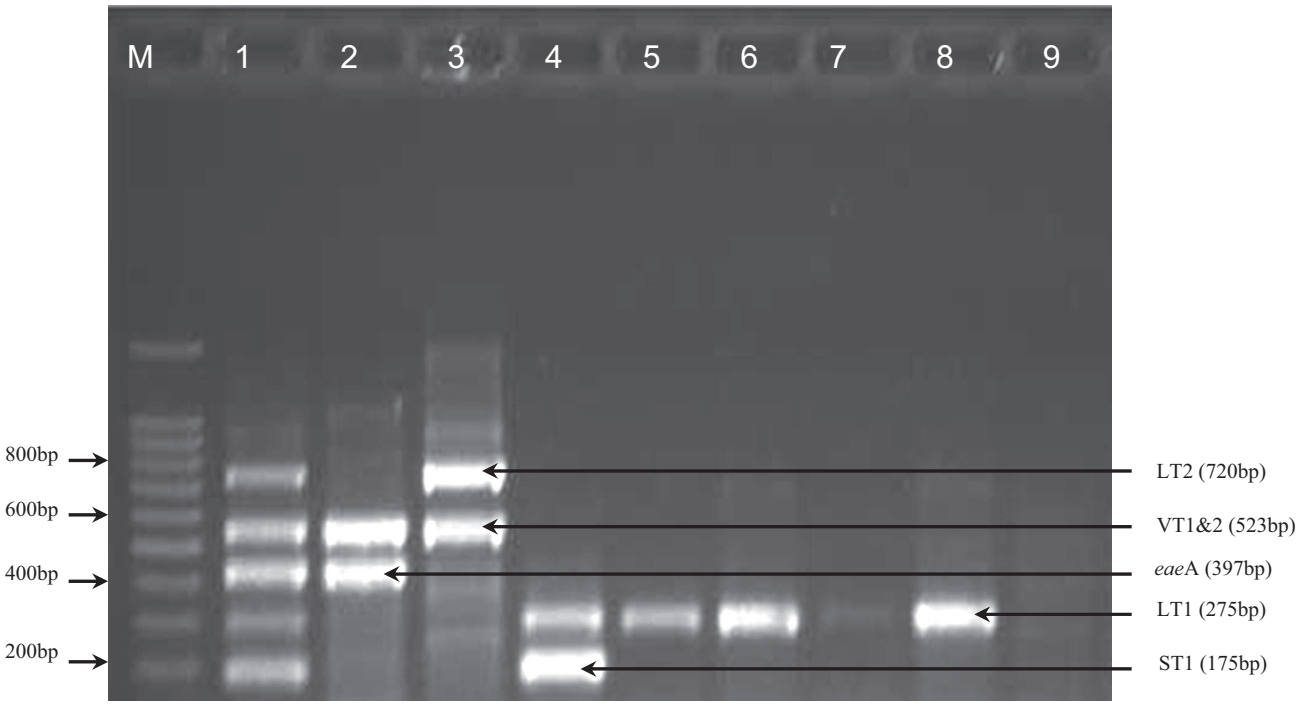

Figure 1. Representative gel showing multiplex PCR for toxigenic genes of *E. coli*. Lane M, 100bp DNA ladder; Lane 1, positive control (DNA mixture of all three reference strains); Lane 2, *E. coli* 0157; Lane 3, *E. coli* SA53; Lane 4, *E. coli* ATCC 35401; Lane 5 to 8, EC15; Lane 9, EC16.

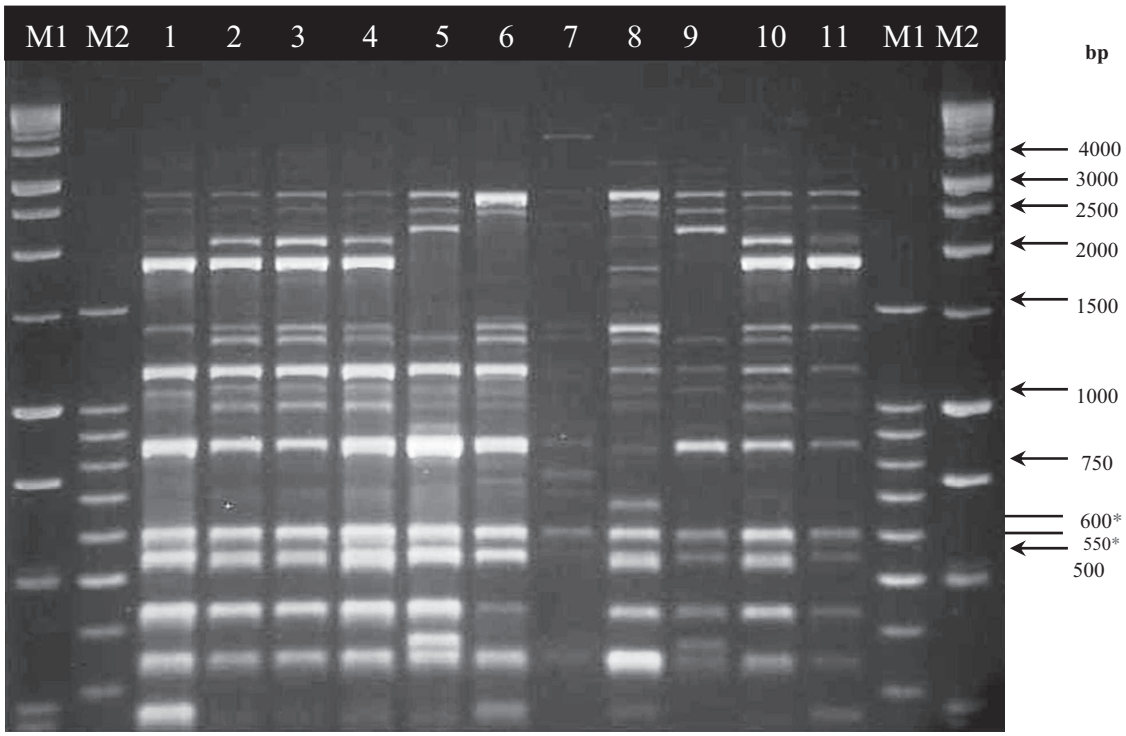

Figure 2. Representative REP-PCR profiles of 10 *E. coli* isolates generated by REP 1R primer. Lane M1: 1kb marker; Lane M2, 100bp marker; Lane 1, EC1; Lane 2, EC2; Lane 3, EC4; Lane 4, EC5; Lane 5, EC6; Lane 6, EC7; Lane 7, EC8; Lane 8, EC 10; Lane 9, EC 11; Lane 10, EC 12; Lane 11, EC13. The consistent bands at 550bp and 600bp (marked in red asterisk) were observed in all the *E. coli* strains.

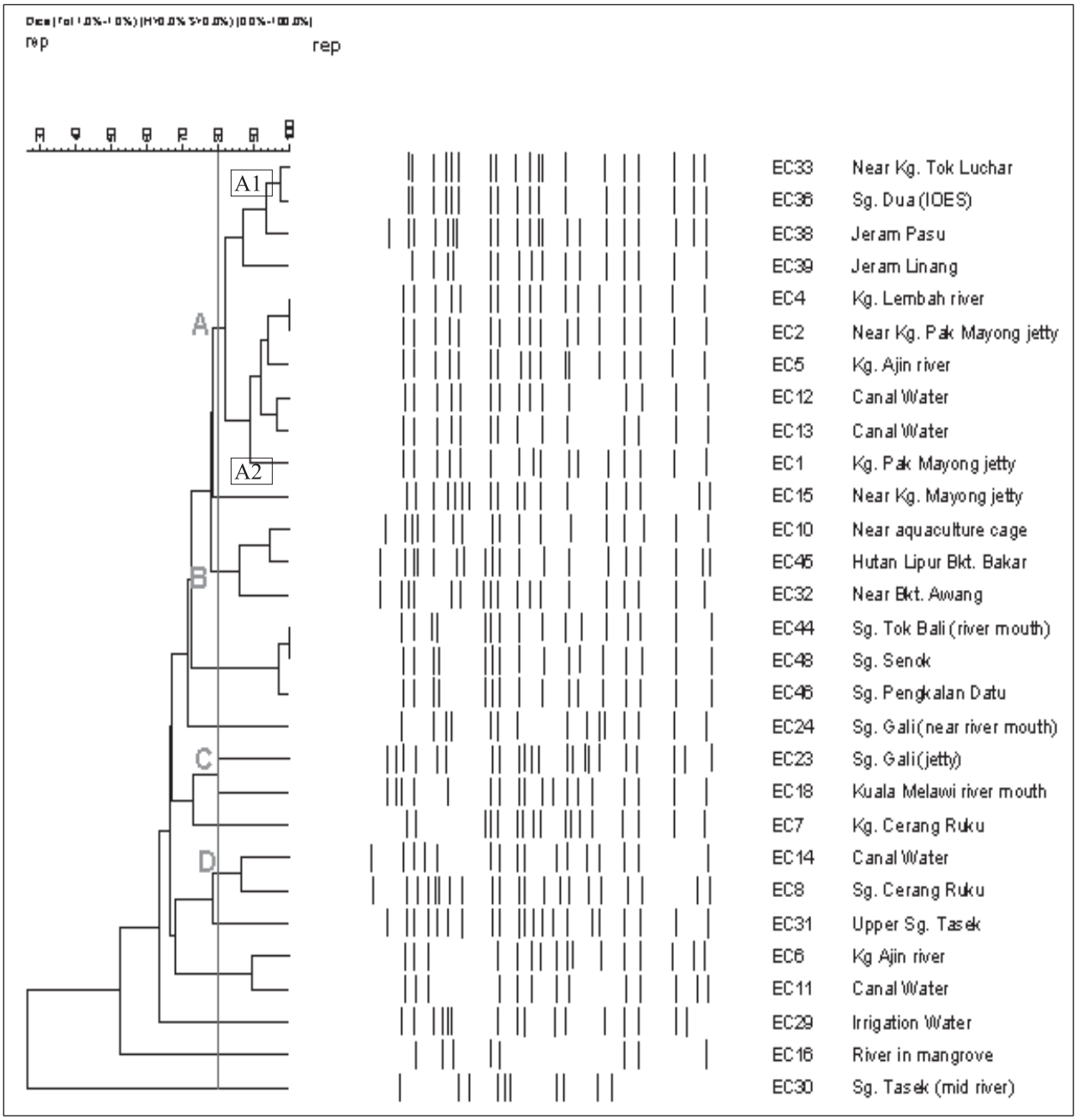

Figure 3. Dendrogram showing the results of cluster analysis of the REP-PCR profiles from *E. coli* isolates generated with GelCompar software by the UPGMA method. The different patterns and location of samples are indicated.

number of antimicrobial agents compared with isolates from human excretions and surface water [18]. Livestock operations and human seepage is the main source for the contamination of surface water with resistant bacteria [18]. Another study showed that livestock contributed more than humans to fecal coliform contamination of surface water and that reducing livestock access to surface water reduced the fecal coliform levels by an average of 94% [19]. However, in this study antimicrobial resistance pattern was not a useful marker as most of the isolates were sensitive to the tested antibiotics.

REP-PCR

REP-PCR subtyped the 116 *E. coli* isolates into 27 DNA patterns (F=0.26-1.0). Repeat isolates from the same water samples showed indistinguishable patterns, indicating the stability of isolates. The REP-

PCR profiles were reproducible and the multiple DNA patterns showed that the *E. coli* isolates were genetically diverse. Multiple DNA fragments in sizes ranging from 300bp to 4kb were observed. All the *E. coli* isolates produced two common DNA bands of sizes, 550bp and 600bp. A representative REP-PCR profile of *E. coli* isolates from 29 water samples from different localities is shown in Figure 2. Isolates that were from sampling sites in the same vicinity had highly similar profiles.

Cluster analysis was carried on the representative REP-PCR profiles of non-repeat *E. coli* isolates and showed 4 separate clusters (A, B, C and D) based on a similarity coefficient of 80% (Figure 3). Cluster A which was the major cluster was subdivided into two subclusters (A1 and A2). Subcluster A1 consisted of 4 isolates from waterfall and river waters. Subcluster A2 included isolates collected around the site Kampung Pak Mayong. The isolates of cluster A were from locations where the waterways were interconnected. Thus, clustering of these isolates was probably due to the movement of strains in these interconnected waterways. Kon *et al*. (2007) reported that different clusters of strains from the same sampling site were closely related due to deposition of *E. coli* from a single source [20]. The ETEC isolate (EC15) was grouped in cluster A as this cluster consisted of isolates retrieved around the jetty area. Furthermore, isolates from the different sources in the same vicinity were clustered together such as canal water samples and lake water samples in cluster D and cluster A. This indicates that there are similarities in the REP profiles of isolates from the same sources. Two isolates from different places, Sungai Tok Bali and Sungai Senok were indistinguishable as they harbored the same profile. Isolates from irrigation water, river water in mangroves and Sungai Tasek had unique REP profiles and were in different cluster. Overall, analysis of the dendrogram showed that the REP-PCR method successfully distinguished the isolates. According to Hahm *et al*. (2003), REP-PCR is a relatively easy and convenient method as compared to AFLP or PFGE [21]. As it is true for all PCR based methods, optimal conditions must be used to ensure reproducibility of data [22]. REP-PCR fingerprinting is a simple, cost effective screening tool for rapid determination of *E. coli* isolates identity and source tracking of fecal contamination of surface water [23].

CONCLUSIONS

Overall, *E. coli* isolates in aquatic environments of Bachok, Kelantan were genetically diverse and heterogeneous. The incidence of pathogenic *E. coli* was low. REP-PCR is a promising molecular method for determining the genomic diversity of environmental *E. coli* strains. However the small sample size was a drawback since the data could not represent the true diversity of *E. coli* in the selected environment. Antimicrobial susceptibility test was not a useful marker in this study compared to molecular method as majority of *E. coli* were susceptible strains. In conclusion, the abundance of *E. coli* in aquatic environments in Bachok, Kelantan indicates that understanding the dynamics of the microbes in the environment is important to improve the bacterial water quality and avoid threats to public health from contaminated water.

ACKNOWLEDGEMENTS

This work was supported by University of Malaya through the PPP grant (FS318/2008AP) and student research grant (P0096/2008C) to Rathi. The authors thank Tim Chuan Seet, Vimala Balasubramaniam, and Lim Sin Yun for technical assistance during the field sampling in Bachok, Kelantan. We thank Prof Dr Richard Kong for the kind gifts of positive control strains.

REFERENCES

1. Lipp, E. K., Farrah, S. A. and Rose, J. B. (2001) Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin* **42**: 286-293.
2. Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R. and Lukasik, J. (2002) Microbial source tracking: current methodology and future directions. *Applied Environmental Microbiology* **68**: 5796–5803.
3. Liu, Y., Debroy, C. and Framico, P. (2007). Sequencing and analysis of the *Escherichia coli* serogroup 0117, 0126, and 0146 O-antigen gene cluster and development of PCR assays targeting serogroup 0117-, 0126-, and 0146- specific DNA sequences. *Molecular and Cellular Probes* **21**: 295-302.
4. Smith, J. L. and Framico, P. M. (2005). Diarrhea- inducing *Escherichia coli*. *In*: P.M. Fratamico, P. M., Bhunia, A. K. and Smith, J. L. (Eds.), *Food borne pathogens microbiology and molecular biology*. United Kingdom, Norfolk: Caister Academic Press, p. 357-382.
5. Safurah, H. J., Raili H. S., Kamaliah, M. N, Fauziah Z. E. and Siong, L. F. (2007). Primary health care key to intersectoral action for health

PCR profiles were reproducible and the multiple DNA patterns showed that the *E. coli* isolates were genetically diverse. Multiple DNA fragments in sizes ranging from 300bp to 4kb were observed. All the *E. coli* isolates produced two common DNA bands of sizes, 550bp and 600bp. A representative REP-PCR profile of *E. coli* isolates from 29 water samples from different localities is shown in Figure 2. Isolates that were from sampling sites in the same vicinity had highly similar profiles.

Cluster analysis was carried on the representative REP-PCR profiles of non-repeat *E. coli* isolates and showed 4 separate clusters (A, B, C and D) based on a similarity coefficient of 80% (Figure 3). Cluster A which was the major cluster was subdivided into two subclusters (A1 and A2). Subcluster A1 consisted of 4 isolates from waterfall and river waters. Subcluster A2 included isolates collected around the site Kampung Pak Mayong. The isolates of cluster A were from locations where the waterways were interconnected. Thus, clustering of these isolates was probably due to the movement of strains in these interconnected waterways. Kon *et al*. (2007) reported that different clusters of strains from the same sampling site were closely related due to deposition of *E. coli* from a single source [20]. The ETEC isolate (EC15) was grouped in cluster A as this cluster consisted of isolates retrieved around the jetty area. Furthermore, isolates from the different sources in the same vicinity were clustered together such as canal water samples and lake water samples in cluster D and cluster A. This indicates that there are similarities in the REP profiles of isolates from the same sources. Two isolates from different places, Sungai Tok Bali and Sungai Senok were indistinguishable as they harbored the same profile. Isolates from irrigation water, river water in mangroves and Sungai Tasek had unique REP profiles and were in different cluster. Overall, analysis of the dendrogram showed that the REP-PCR method successfully distinguished the isolates. According to Hahm *et al*. (2003), REP-PCR is a relatively easy and convenient method as compared to AFLP or PFGE [21]. As it is true for all PCR based methods, optimal conditions must be used to ensure reproducibility of data [22]. REP-PCR fingerprinting is a simple, cost effective screening tool for rapid determination of *E. coli* isolates identity and source tracking of fecal contamination of surface water [23].

CONCLUSIONS

Overall, *E. coli* isolates in aquatic environments of Bachok, Kelantan were genetically diverse and heterogeneous. The incidence of pathogenic *E. coli* was low. REP-PCR is a promising molecular method for determining the genomic diversity of environmental *E. coli* strains. However the small sample size was a drawback since the data could not represent the true diversity of *E. coli* in the selected environment. Antimicrobial susceptibility test was not a useful marker in this study compared to molecular method as majority of *E. coli* were susceptible strains. In conclusion, the abundance of *E. coli* in aquatic environments in Bachok, Kelantan indicates that understanding the dynamics of the microbes in the environment is important to improve the bacterial water quality and avoid threats to public health from contaminated water.

ACKNOWLEDGEMENTS

This work was supported by University of Malaya through the PPP grant (FS318/2008AP) and student research grant (P0096/2008C) to Rathi. The authors thank Tim Chuan Seet, Vimala Balasubramaniam, and Lim Sin Yun for technical assistance during the field sampling in Bachok, Kelantan. We thank Prof Dr Richard Kong for the kind gifts of positive control strains.

REFERENCES

1. Lipp, E. K., Farrah, S. A. and Rose, J. B. (2001) Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin* **42**: 286-293.
2. Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R. and Lukasik, J. (2002) Microbial source tracking: current methodology and future directions. *Applied Environmental Microbiology* **68**: 5796–5803.
3. Liu, Y., Debroy, C. and Framico, P. (2007). Sequencing and analysis of the *Escherichia coli* serogroup 0117, 0126, and 0146 O-antigen gene cluster and development of PCR assays targeting serogroup 0117-, 0126-, and 0146- specific DNA sequences. *Molecular and Cellular Probes* **21**: 295-302.
4. Smith, J. L. and Framico, P. M. (2005). Diarrhea- inducing *Escherichia coli*. *In*: P.M. Fratamico, P. M., Bhunia, A. K. and Smith, J. L. (Eds.), *Food borne pathogens microbiology and molecular biology*. United Kingdom, Norfolk: Caister Academic Press, p. 357-382.
5. Safurah, H. J., Raili H. S., Kamaliah, M. N, Fauziah Z. E. and Siong, L. F. (2007). Primary health care key to intersectoral action for health